The dual role of the X-linked FoxP3 gene in human cancers

Redpath, Margaret; Xu, Bin; van Kempen, Leon C; Spatz, Alan

Published in:
Molecular oncology

DOI:
10.1016/j.molonc.2011.03.001

Publication date:
2011

MOLECULAR ONCOLOGY 5 (2011) 156–163

available at www.sciencedirect.com

www.elsevier.com/locate/molonc

Review

The dual role of the X-linked *FoxP3* gene in human cancers

Margaret Redpath[a], *Bin Xu*[a], *Leon C. van Kempen*[a,b], *Alan Spatz*[a,b,*]

[a]*Department of Pathology, McGill University, Montreal, QC, Canada*
[b]*Lady Davis Medical Research Institute, Montreal, QC, Canada*

ARTICLE INFO

Article history:
Received 22 February 2011
Accepted 11 March 2011
Available online 30 March 2011

Keywords:
Melanoma
Fox3P
T-lymphocyte-associated antigen 4 (CTLA-4)/ipilimumab

ABSTRACT

The *FoxP3* (forkhead box P3) gene is an X-linked gene that is submitted to inactivation. It is an essential transcription factor in $CD4^+CD25^+$FoxP3 regulatory T cells, which are therapeutic targets in disseminated cutaneous melanoma. Moreover, *FoxP3* is an important tumor suppressor gene in carcinomas and has putative cancer suppressor gene function in cutaneous melanoma as well. Therefore understanding the structure and function of the *FoxP3* gene is crucial to gaining insight into the biology of melanoma to better develop immunotherapeutics and future therapeutic strategies.

After decades of continuous failures, ipilumimab – which blocks cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) was recently demonstrated to improve overall survival in patients with previously treated metastatic cutaneous melanoma. Ipilimumab, with or without a gp100 vaccine, was associated with improved survival as compared with gp100 alone (Hodi et al., 2010). This is the first ever reported positive phase 3 trial in advanced melanoma. Ipilumimab blocks CTLA-4 and this blockade may improve survival by modifying the intratumoral effector/regulatory cell ratio, thus highlighting the importance of regulatory T-lymphocytes (T_{Reg}) for melanoma progression *in vivo*. $CD4^+$ T_{Reg} express the transcription factor forkhead box P3 (Foxp3), which is essential for their normal development and function (Fontenot and Rudensky, 2005). Antigenic stimulation of conventional $CD4^+$ T cells in the presence of transforming growth factor-β (TGF-β) induces FoxP3 expression and the acquisition of suppressor function (Amarnath et al., 2007; Chen and Konkel, 2010; Fantini et al., 2004; Floess et al., 2007; Fu et al., 2004; Horwitz et al., 2008; Huber et al., 2009; Huter et al., 2008; Kawamoto et al., 2010; Marie et al., 2005; Samanta et al., 2008; Schramm et al., 2004; Zheng et al., 2006; Zhou et al., 2008a, 2010). It has been clearly established that stable FoxP3 expression is required to maintain suppressive properties of T_{Reg} cells (Blache et al., 2009). Although it is necessary for the continued suppressive action of functional T_{Reg} cells, FoxP3 expression alone is not sufficient to accurately identify functional T_{Reg} cells because post-translational changes in discrete residues of the protein can lead to loss of function (Li and Greene, 2008). The regulation of FoxP3 expression is essential to modulating immune surveillance through the regulatory T-cell lineage. The *FoxP3* gene has a dual role as it is also an X-linked tumor suppressor

* *Corresponding author*. Department of Pathology, Jewish General Hospital, 3755 Cote Ste Catherine, Montreal, QC H3T 1E2, Canada. Tel.: +1 514 340 8222 3262.
E-mail address: alan.spatz@mcgill.ca (A. Spatz).
1574-7891/$ – see front matter © 2011 Federation of European Biochemical Societies. Published by Elsevier B.V. All rights reserved.
doi:10.1016/j.molonc.2011.03.001

gene (TSG) in several solid tumors. Whether it is a TSG in melanoma has yet to be determined. Understanding the regulation and function of the *FoxP3* gene is therefore crucial to better understanding the biology of melanoma.

1. Structure of the *FoxP3* gene and regulation by transcription factors

The *FoxP3* gene is located on the X chromosome (chr) at Xp11.23 and is submitted to X chr inactivation (Bennett et al., 2001; Wang et al., 2009). The gene contains 11 coding exons (exons 1–11) and 3 non coding exons (Bennett et al., 2001). The two 5′ non coding exons (−2a and −2b) are spliced into a common non coding exon (−1) (Floess et al., 2007; Kaur et al.,2 010; Smith et al., 2006). The −2b and −1 exons encompass regulatory *cis*-elements. (Lal and Bromberg, 2009; Lopes et al., 2007). It should be noticed that several reports refer to exon −1 as exon 1 and name the exons differently from 1 to 12. An AA insertion in exon 8 leads to the *scurfy* phenotype in mice. (Bennett et al., 2001). About 60% of patients with an *IPEX* syndrome-immune dysfunction, polyendocrinopathy, enteropathy, X-linked syndrome have missense mutations in exons 9, 10, and 11 which encode the forkhead domain (Bennett et al., 2001; Harbuz et al., 2010; Owen et al., 2003; Rubio-Cabezas et al., 2009; Torgerson et al., 2007).

The FoxP3 protein is highly conserved (Lal et al., 2009; Sadlon et al., 2010; Zheng et al., 2010). It is critical to the understanding of FoxP3 function to realize that human cells express three FoxP3 isoforms (Aarts-Riemens et al., 2008; Allan et al., 2005; Kaur et al., 2010; Smith et al., 2006; Ziegler, 2006) The longest form resembles the murine FoxP3, whereas the other two are unique to humans. FoxP3ΔE2 lacks exon 2, which is part of the repressor domain in the FoxP3 protein. Compared to full length FoxP3, expression of FoxP3ΔE2 in human $CD4^+CD25^-FoxP3^-$ T cells leads to more IL-2 secretion and proliferation in response to T-cell receptor (TCR) stimulation (Allan et al., 2005). It has been proposed that FoxP3ΔE2 acts as a dominant negative isoform (Li et al., 2007; Xu et al., 2010). Human T_{Regs} can also express a third isoform that lacks both exon 2 and exon 7 (Kaur et al., 2010). The absence of exon 7, which encodes a leucine zipper motif, in the FoxP3ΔE2Δ7 isoform abrogates the suppressive function of T_{Regs} (Kaur et al., 2010). This emphasizes the importance of distinguishing the FoxP3, FoxP3ΔE2, and FoxP3ΔE2Δ7 isoforms to accurateley sub-type T_{Regs}.

Conservative noncoding sequences (CNS) are located within 500 base pairs 5′ to the transcription start site and serve as a proximal promoter region (Zhang et al., 2008). It contains TATA, GC, and CAAT boxes that, when mutated, lead to decreased FoxP3 activity (Mantel et al., 2006). This region also contains binding sites for important transcription factors, such as nuclear factor of activated T cells (NFAT), activator protein-1 (AP-1), and Sp-1 that play a role in anti-CD3/CD28-driven FoxP3 expression (Kim, 2009; Zheng et al., 2010). The 5′ intron 2, located between exon −2a and exon −1, contains a CNS and is referred to as the intronic enhancer, whereas the CNS situated 5 kb upstream of the transcriptional start site is referred to as the upstream enhancer (Lal et al., 2009). The proximal promoter is able to bind with STAT5 and is dependent on IL-2Rβ signalling (Burchill et al., 2007). It has been recently suggested that STAT5 is required for FoxP3 expression (Burchill et al., 2007). The second enhancer region (3′ CNS enhancer) locates to +4301 to +4500 as identified by luciferase reporter assays in Jurkat cells (Wu et al., 2006). This enhancer region contains a CREB/ATF (activating transcription factor) motif that is capable of binding CREB and maintaining FoxP3 expression and thus a stable T_{Reg} population (Kim and Leonard, 2007). Binding sites for STAT5 and STAT3 that mediate signalling of inflammatory cytokines, including IL-2, IL-6, IL-21, and IL-27, are also located within this enhancer region (Zorn et al., 2006). It seems that both STAT5 and STAT3 bind to the same target site, while STAT3 binds with a much lower affinity. Recently, using mice with specific deletions of the conservative regions, Zheng et al. have identified another pioneer CNS (Zheng et al., 2009). It locates to intron 4, which is between exons 1 and 2, binds c-Rel, and acts to potently increase the number of T_{Regs} in the thymus and periphery.

In Th1, the IFN-γ–induced protein interferon regulatory factor 1 binds to the FoxP3 proximal promoter and inhibits FoxP3 expression (Ouaked et al., 2009). In Th2, IL-4 inhibits FoxP3 expression in peripheral naive $CD4^+CD25^-$ T cells by stimulating phosphorylation of STAT6, which binds between exons −2b and −1 and inhibits TGF-β-induced FoxP3 expression (Takaki et al., 2008). TGF-β and IL-4 cosignalling induces IL-9 secretion, leading to the newly identified $IL\text{-}9^+IL\text{-}10^+FoxP3^-$ (Th9) subset (Vang et al., 2008).

Polymorphisms of the *FoxP3* gene, including single nucleotide polymorphisms (SNP) and microsatellite polymorphisms have been reported in multiple case-control and cohort studies, mostly in patients with autoimmune diseases or cancers and their controls (Lin et al., 2011). Reported polymorphisms include SNPs in the promoter region, SNPs in the intron regions, SNPs downstream of the coding regions, and microsatellite polymorphism (GT)n in the promoter region, and TC(n) in the intron region (Lan et al., 2010). While most of the polymorphisms seem to be clinically irrelevant, a few alleles do show weak clinical correlation with autoimmune diseases, including SNPs in primary biliary cirrhosis and psoriasis, and (TC)n microsatellite polymorphisms in autoimmune thyroid disease (Inoue et al., 2010; Wang et al., 2010a). Overall, there is a lack of convincing evidence to suggest that any FoxP3 polymorphism is clinically significant.

2. Epigenetic regulation of *FoxP3* expression

It is well-established that methylation of CpG sequences inhibits acetylation of histones and binding of transcription factors to DNA, thus resulting in quiescent genes. Demethylation of CpG sequences and acetylation of histones, on the other hand, are features of active genes. Such epigenetic modifications are also observed in the *FoxP3* gene. The methylation status of the CpG residues in the proximal promoter region plays an essential role in FoxP3 expression (Janson et al., 2008). Demethylation induced by treatment with 5-aza-2′-deoxycytidine (Aza) leads to FoxP3 expression in human NK cells (Lal et al., 2009; Moon et al., 2009). Ten to forty-five percent of the CpG sites in the FoxP3 proximal

promoter are methylated in naive $CD4^+CD25^-$ T cells, whereas all are demethylated in natural T_{Regs} (Lal and Bromberg, 2009; Polansky et al., 2008) TGF-β induces demethylation of CpG at this site in $CD4^+CD25^-$ T cells (Lal et al., 2009). Multiple CpG sequences have been identified in the *FoxP3* gene, from 5′ to 3′ as follows: i) within an upstream CNS, 5–6000 bp 5′ to the transcription starting site; ii) within the intronic enhancer, and iii) within intron 10, between exons 7 and 8 (Floess et al., 2007; Hansmann et al., 2010; Lal et al., 2009; Polansky et al., 2010; Zheng et al., 2010). The intronic enhancer contains a Runx1 binding sequence. When the enhancer is demethylated (i.e. activated), it can be occupied by a FoxP3, Runx1, and core-binding factor-β complex (Zhang et al., 2008).

TGF-β induces FoxP3 expression in peripheral naive $CD4^+CD25^-$ T cells. In addition to TGF-β receptor–induced SMAD3 signaling for T_{Reg} generation, TGF-β signaling may also act via TIEG1 and the E3 ubiquitin ligase *itch* in a ubiquitin-dependent pathway (Venuprasad et al., 2008). TGF-β also inhibits the phosphorylation of ERK leading to inhibition of DNA methyltransferase (DNMT) expression; and inhibition of DNMT with siRNA or DNMT inhibitors leads to FoxP3 expression in $CD4^+$ T cells, suggesting that inhibition of DNMT activity plays an important role in FoxP3 expression (Luo et al., 2008).

The inflammatory cytokine IL-6 suppresses the development and function of $T_{Regs.}$ (Lal et al., 2009). Actually, IL-6 both induces DNMT1 expression and enhances its activity. IL-6 induces STAT3-dependent methylation of the upstream FoxP3 enhancer by DNMT1 in T_{Regs}, leading to repression of FoxP3 (Wang et al., 2007). Preactivated $CD4^+CD25^-$ T cells or $CD4^+CD25^-CD44^{hi}$ memory T cells express very little FoxP3 after TGF-β stimulation. This is probably the result of high levels of DNMT1 activity in these cells because inhibition of DNMT with Aza or deficiency of DNMT1 in T cells leads to FoxP3 expression, suggesting that regulation of FoxP3 is tightly controlled by epigenetic modifications in activated $CD4^+$ T cells (Lal et al., 2009).

Natural T_{Regs} possess demethylated CpGs at the *FoxP3* locus and show stable *FoxP3* expression, whereas TGF-β-induced T_{Regs} show methylated CpGs and do not maintain constitutive *FoxP3* expression after restimulation in the absence of TGF-β (Baron et al., 2007; Floess et al., 2007; Lal and Bromberg, 2009; Polansky et al., 2008). It has been reported that a fraction of the $FoxP3^+CD4^+$ natural T_{Regs} adoptively transferred into lymphopenic mice are converted into $FoxP3^-$ T cells (Bruinsma et al., 2010) http://www.ncbi.nlm.nih.gov/pubmed/19174509. Under inflammatory conditions, $FoxP3^+$ T_{Regs} lose FoxP3 expression and thus suppressive function in an IL-6–dependent manner (Lal et al., 2011) IL-6 and TCR signalling induce down-regulation of FoxP3 expression and lead to development of Th17 cells (Maitra et al., 2009) http://www.ncbi.nlm.nih.gov/pubmed/18585065. One mode of action of IL-6 is to induce remethylation of CpG DNA at the upstream enhancer and thus down-regulates FoxP3 expression in natural T_{Regs} (Lal et al., 2009). Epigenetic inheritance during the cell cycle is crucial in maintaining chromatin structure in cell lineages. But the extrinsic and intrinsic signals that regulate CpG DNA methylation and perpetuate the H3 methylation level at the *FoxP3* locus from one cell cycle to another are not yet understood.

3. Mechanisms of impaired regulation by $CD4^+CD25^+FoxP3^+$ regulatory T cells in human autoimmune diseases

T_{Reg} cells, defined by the expression of CD4, CD25 and FoxP3, have a central role in protecting an individual from autoimmunity. This role was first identified in mice in which the absence or depletion of T_{Regs} resulted in the development of autoimmune gastritis, thyroiditis, diabetes and inflammatory bowel disease (IBD) (Ochs et al., 2007). Subsequently, numerous studies in animal models of autoimmunity showed that defects in $CD4^+CD25^+FoxP3^+$ T_{Regs} contribute to the development of autoimmunity and that the disease could be reversed by the adoptive transfer of T_{Regs} (Buckner, 2010). The importance of T cell regulation in human disease is highlighted by the severe inflammation and autoimmunity that occurs in individuals who suffer from IPEX. These individuals develop a broad range of autoantibodies causing insulin-dependent diabetes, thyroiditis, eczema, hemolytic anemia and IBD. In the absence of a bone marrow transplant, IPEX patients die at an early age (Wildin and Freitas, 2005).

In mouse models, the concept that inadequate numbers of T_{Regs} may contribute to autoimmunity is supported by the occurrence of aggressive autoimmunity in scurfy mice and is indirectly implied by the successful treatment of autoimmunity in mice through the adoptive transfer of wild-type T_{Regs} (Komatsu and Hori, 2007). In addition, there is evidence from mouse models that, under the appropriate conditions, T_{Regs} can be induced in the periphery, and that these T_{Regs} may prevent the development of autoimmunity. Multiple factors influence the homeostasis and induction of T_{Regs} in the periphery, including CD28, IL-2, TGF-β and dendritic cells (DCs) (Buckner, 2010; Buckner and Ziegler, 2008).

Evidence that an inadequate number of T_{Regs} leads to autoimmunity in humans is most clearly shown in patients with IPEX, who completely lack T_{Regs} as a result of a mutation in *FoxP3* (Lal and Bromberg, 2009). However, most patients with autoimmune disease probably have a more modest reduction in T_{Regs}. In these common diseases, the challenge is to determine whether the number of T_{Regs} is inadequate at the site of inflammation and whether this is due to systemic factors or factors in the local tissue milieu (Buckner, 2010). In human disease, the task of enumerating T_{Regs} has been complicated by the presence of multiple T_{Reg} subsets and the lack of a cell marker that is unique to T_{Regs}. Type 1 regulatory T (T_R1) cells are induced in the periphery and suppress T cell proliferation through the production of interleukin-10 (IL-10) and TGF-β. TR1 cells do not have a unique cell marker but are identified by their production of IL-10 and absence of pro-inflammatory cytokines (Roncarolo and Gregori, 2008; Veldman et al., 2009). T helper 3 (T_H3) cells are a regulatory T cell population that originates in the periphery and mediates suppression through the secretion of TGF-β; similar to T_R1 cells, they do not have a unique cell surface marker (Carrier et al., 2007). $CD4^+CD25^+FoxP3^+$ T_{Regs} can be divided into two groups: thymus-derived natural T_{Reg} cells and periphery-induced adaptive T_{Reg} cells. Both populations express FoxP3 and suppress immune responses through contact-dependent mechanisms and the production of soluble factors, including

the cytokines TGF-β, IL-10 and IL-35 (Buckner, 2010). Thymus-derived $CD4^+CD25^+FoxP3^+$ T_{Reg} cells are stable with respect to retaining regulatory function and FoxP3 expression in the periphery. They are unique in that their *FoxP3* locus is demethylated and they express the transcription factor Helios (Fujimoto et al., 2011; Thornton et al., 2010). Adaptive T_{Reg} cells can be induced in the periphery from a $CD4^+FoxP3^-$ T cell population following T cell receptor stimulation in the presence of TGF-β (Mahic et al., 2008). It has now become clear that the $FoxP3^+$ T cell population is composed of several populations that are defined by the expression of CD25, CD45RA and FoxP3. Miyara et al. defined these populations as a naive T_{Reg} cell population that is $CD25^{hi}CD45RA^+FoxP3^{hi}$, an effector T_{Reg} cell population that is $CD25^{hi}CD45RA^-FoxP3^{hi}$ and a non-regulatory $FoxP3^+$ population that is $CD25^{hi}CD45RA^-FoxP3^{low}$ (Miyara and Sakaguchi, 2011; Miyara et al., 2009). Another difficulty is the extent to which the peripheral blood reflects the global number of T_{Regs} in the body and, more specifically, their number in inflamed tissues.

T_{Regs} were first defined on the basis of their expression of CD25, which forms part of the high-affinity IL-2 receptor. Unfortunately, the definition of T_{Regs} based on the level of CD25 expression has not been consistently reported in the literature, making comparisons between studies difficult. Furthermore, CD25 is also expressed by recently activated T cells, resulting in the inclusion of $CD4^+CD25^+$ effector T cells in the T_{Reg} population (Buckner, 2010). With the discovery that expression of FoxP3 plays a central role in the differentiation and maintenance of T_{Regs}, the use of flow cytometry-based analysis of FoxP3 expression in T cells became the gold standard for defining T_{Regs}. However, it then became evident that FoxP3 can also be expressed by effector T cells following activation, thus any assessment of T_{Regs} number or function is likely tainted by inclusion of recently activated effector T cells in the T_{Regs} population. Furthermore, as FoxP3 is a nuclear protein, assessment of its expression in T cells requires fixation and permeabilization of the cells, resulting in an inability to obtain viable cells for further functional analysis. In the past few years, additional markers, such as CD127 have been identified that assist in the distinction of effector T cells from T_{Regs} and facilitate the experimental purification of T_{Regs} (Simonetta et al., 2010).

In the context of anti-CTLA-4 melanoma therapy, a major challenge is to measure resistance of effector T cells to suppression. The resistance of effector T cells to T_{Regs} has been observed in several animal models of autoimmunity (Chen et al., 2010; Schneider et al., 2008). In these models, inflammation and tissue destruction progress despite the presence of functional T_{Regs} at the site of inflammation. Such findings suggest that a resistance of effector T cells to T_{Regs} may contribute to autoimmunity. Whether this might be of interest to predict response to anti-CTLA-4 therapy needs further investigation.

4. *FoxP3* as an X-linked tumor suppressor gene

Foxp3 is expressed in epithelial cells from various organs such as breast, thymus, prostate and lung. Importantly, mice that are heterozygous for *FoxP3* mutations spontaneously develop mammary carcinomas at a high frequency (Chen et al., 2008). Genetic analyses in both mice and humans revealed that *Foxp3* is an important X-linked tumor suppressor in breast and in prostate cancer (Gupta et al., 2007; Karanikas et al., 2008; Katoh et al., 2010; Kiniwa et al., 2007; Ladoire et al., 2011; Li et al., 2011; Liu and Zheng, 2007; Mahmoud et al., 2010; Merlo et al., 2009; Valdman et al., 2010; Wang et al., 2009, 2010b; Yokokawa et al., 2008; Zuo et al., 2007a, 2007b). Mice with germline *FoxP3* mutations are substantially more prone to developing both spontaneous and carcinogen-induced mammary carcinomas (Zuo et al., 2007b). The role of the *FoxP3* gene in mammary carcinogenesis has been supported by several lines of evidence. The *FoxP3* gene is expressed in normal breast epithelia but is down-regulated in mammary cancer. Ectopic expression of *FoxP3* in a variety of breast cancer cell lines resulted in cell cycle arrest and cessation of cell growth (Zuo et al., 2007a). Moreover, FoxP3 directly regulates transcription of *ErbB2*, *Skp2* and *CDKN1A* (p21) (Katoh et al., 2010). Frequent chromosomal deletions and somatic mutations of the *FoxP3* gene were detected in human cancer samples including cutaneous melanomas (Fujii et al., 2010; Karanikas et al., 2008;). There is a down-regulation of FoxP3 protein in cancer cells compared to normal breast epithelia. The *FoxP3* gene also plays an important role in prostate epithelia. Among human prostate cancers, frequent chromosomal deletions, somatic mutations and epigenetic silencing of the *FoxP3* gene were found (Li et al., 2011; Wang et al., 2009, 2010b). Since the *FoxP3* gene is located on the X chromosome, a genetic/epigenetic single-hit results in inactivation of this gene in males, escaping the Knudson model (Spatz et al., 2004). Immunohistochemistry revealed that FoxP3 expression is significantly down-regulated in cancer cells when compared to normal prostate glands (Valdman et al., 2010). Moreover, mice with prostate-specific ablations of FoxP3, $FoxP3^{fl/y}$; $PB\text{-}Cre^+$, developed prostatic hyperplasia and prostatic intraepithelial neoplasm (PIN) that are putative pre-cancerous lesions of the prostate (Ebelt et al., 2009; Wang et al., 2009). In human samples, FoxP3 expression in PINs are down-regulated compared to adjacent normal prostate glands, which suggests that the inactivation of the *FoxP3* gene plays an important role in the initial stage of prostatic carcinogenesis (Ebelt et al., 2009).

Another interesting aspect of FoxP3 abnormalities is that some types of cancers predominantly express splice variants of the FoxP3 protein in addition to those occurring in non-transformed cells (FoxP3, FoxP3ΔE2, and FoxP3ΔE2Δ7). In cutaneous melanomas, and in some breast and ovarian cancers, and malignant T cells of Sezary syndrome, specific splice variants of the FoxP3, such as ΔE3, ΔE3–4, ΔE3/8 and ΔE8, were reported to be preferably expressed (Kaur et al., 2010; Smith et al., 2006) The Δ3–4 splice variant results in a truncated FoxP3 with a premature stop codon, and therefore might contribute to the malignant progression of cells (Wang et al., 2009). Whereas FoxP3 has been shown to up-regulate CTLA-4 expression, it is not known whether different variants in the primary melanoma are associated with different responses to ipilimumab at a later stage.

Expression of the oncogene c-MYC has been demonstrated to be directly repressed by FoxP3 in prostate epithelia (Wang et al., 2009). Overexpression of c-MYC contributes to more aggressive and poorly differentiated cancer phenotypes and has been involved in the biology of melanoma. c-MYC is a

sequence-specific transcription factor and an important player in various cellular processes including cell cycle and apoptosis; processes which are also dysregulated in cancer cells with high c-MYC expression levels. C-MYC directly activates *CDK4* and *CCND2* expression, while indirectly repressing CDK inhibitors such as *CDKN1A* (*p21*) and *CDKN2B* (*p15*) expression (Wang et al., 2011). Moreover, c-MYC directly upregulates *eIF4E* and *eIF2α*; both of which are the rate-limiting effectors of cell cycle.

HER2 is a member of the transmembrane receptor tyrosine kinases and is involved in the regulations of various cellular functions such as cell growth and survival. The cytoplasmic portion of HER2 is phosphorylated at conserved tyrosine residues and these phosphorylated tyrosines can serve as binding sites for adapters which link HER2 to its downstream pathways or targets such as PI3K-Akt and MAPK-Erk. Both *HER2* gene amplification and loss of nuclear *FoxP3* contribute to HER2 overexpression in breast cancer samples (Mahmoud et al., 2010; Zhou et al., 2008b). FoxP3 can repress transcription of HER2 in human breast cancers by binding directly to the *ERBB2* gene promoter (Zhou et al., 2008b). Since *in vitro* HER2 overexpression nullifies the ability of FoxP3 to inhibit cell growth, repression of HER2 may be critical for the tumor suppressor function of FoxP3 in the breast epithelial cells (Zuo et al., 2007a). Maybe this can partially explain why circulating $CD4^+CD25^+FoxP3^+$ T_{Regs} decrease in breast cancer patients after vaccination with a modified MHC class II HER2/*neu* peptide (Gates et al., 2010). Recently it has been demonstrated that the influence of FoxP3 was dependent on the molecular sub-type of breast cancer. Indeed, FoxP3 expression in cancer cells may be a marker of good prognosis in HER2-overexpressing tumors and of poor prognosis in other molecular sub-types of breast cancer (Ladoire et al., 2011).

High levels of expression of SKP2 have been reported in a wide variety of cancers including melanoma (Katagiri et al., 2006; Rose et al., 2011). SKP2 is an important player in the ubiquitin-dependent degradation of $p27^{KIP1}$, a CDK inhibitor especially of Cyclin-E/CDK2 and Cyclin-A/CDK2 (Katagiri et al., 2006; Rose et al., 2011). SKP2 is robustly expressed during S and G2 phases of the cell cycle and regulates p27 degradation, thus facilitating progression of the cell cycle. It has been demonstrated that FoxP3 directly represses *SKP2* expression in human and mouse mammary epithelial cells (Zuo et al., 2007a). FoxP3 occupies the *Skp2* promoter and represses promoter activity of the locus (Zuo et al., 2007a). FoxP3 directly regulates key molecules of cell cycle regulation, which further supports the notion that *FoxP3* is an important tumor suppressor.

Previous reports have revealed that FoxP3 forms complexes with Rel family transcription factors NFAT and NFκB, and FoxP3 blocks their ability to activate *Il-2* and *INFγ* transcription (Ruan et al., 2009; Soligo et al., 2011). By making a repressive FoxP3:NFAT complex, FoxP3 inhibits NFAT:AP-1 complex at the *Il-2* promoter (Kim, 2009). FoxP3 could also weaken the DNA binding activity of AP-1 (Lee et al., 2008). AML1/RUNX1, which activates endogenous *Il-2* and *IFNγ* expression in $CD4^+$ T cells, is reported to make a complex with FoxP3. (Hancock and Ozkaynak, 2009; Ono et al., 2007). AML1/RUNX1 could bind to the *Il-2* enhancer with FoxP3 and exert optimal repression of *Il-2* in T_{Regs} (Ono et al., 2007).

5. Conclusion

The *FoxP3* gene has important functions as a tumor suppressor gene in human carcinomas and recent data suggests it plays a role in melanoma as well. At the same time, this gene directly commands the natural regulatory T cells that have been demonstrated to be an effective target for melanoma therapy. Thus an interesting issue is whether *FoxP3* gene is a friend or a foe for melanoma therapy. Perhaps gaining better insight about the roles of aberrantly spliced variants will be the first step in answering this question. Although molecular mechanisms have not yet been clarified, some agents have been reported to increase FoxP3 in cancer cells. Anisomycin could induce the transcription of *FoxP3* in various breast cancer cell lines, resulting in significantly repressed cell growth *in vitro* and in xenografts *in vivo* (Liu et al., 2009). In breast and colon cancer cell lines, *FoxP3* expression is directly regulated by p53. Doxorubicin, which activates p53, dramatically activates *FoxP3* transcription *in vitro* (Jung et al., 2010). Maybe the restoration of *FoxP3* functions in melanoma cells with low *FoxP3* expression or ΔE3/ΔE3ΔE4 variants combined with anti-CTLA-4 therapy could have potential as a novel therapeutic strategy. Identification of these variants could also be of help in discovering the first predictive biomarker for anti-CTLA-4 therapy. It will also likely enable improved development of effective combinations of immunotherapy and targeted therapy.

REFERENCES

Aarts-Riemens, T., Emmelot, M.E., Verdonck, L.F., Mutis, T., 2008. Forced overexpression of either of the two common human Foxp3 isoforms can induce regulatory T cells from CD4(+) CD25(−) cells. Eur. J. Immunol. 38, 1381–1390.

Allan, S.E., Passerini, L., Bacchetta, R., Crellin, N., Dai, M., Orban, P.C., Ziegler, S.F., Roncarolo, M.G., Levings, M.K., 2005. The role of 2 FOXP3 isoforms in the generation of human $CD4^+$ Tregs. J. Clin. Invest. 115, 3276–3284.

Amarnath, S., Dong, L., Li, J., Wu, Y., Chen, W., 2007. Endogenous TGF-beta activation by reactive oxygen species is key to Foxp3 induction in TCR-stimulated and HIV-1-infected human $CD4^+CD25^-$ T cells. Retrovirology 4, 57.

Baron, U., Floess, S., Wieczorek, G., Baumann, K., Grutzkau, A., Dong, J., Thiel, A., Boeld, T.J., Hoffmann, P., Edinger, M., Turbachova, I., Hamann, A., Olek, S., Huehn, J., 2007. DNA demethylation in the human FOXP3 locus discriminates regulatory T cells from activated FOXP3(+) conventional T cells. Eur. J. Immunol. 37, 2378–2389.

Bennett, C.L., Brunkow, M.E., Ramsdell, F., O'Briant, K.C., Zhu, Q., Fuleihan, R.L., Shigeoka, A.O., Ochs, H.D., Chance, P.F., 2001. A rare polyadenylation signal mutation of the FOXP3 gene (AAUAAA–>AAUGAA) leads to the IPEX syndrome. Immunogenetics 53, 435–439.

Blache, C., Adriouch, S., Calbo, S., Drouot, L., Dulauroy, S., Arnoult, C., Le Corre, S., Six, A., Seman, M., Boyer, O., 2009. Cutting edge: CD4-independent development of functional $FoxP3^+$ regulatory T cells. J. Immunol. 183, 4182–4186.

Bruinsma, M., Wils, E.J., Lowenberg, B., Cornelissen, J.J., Braakman, E., 2010. The impact of CD4(+)Foxp3(+) Treg on immunity to murine cytomegalovirus after bone marrow transplantation depends on the peripheral or thymic source of T cell regeneration. Transpl. Immunol..

Buckner, J.H., 2010. Mechanisms of impaired regulation by CD4(+) CD25(+)FOXP3(+) regulatory T cells in human autoimmune diseases. Nat. Rev. Immunol. 10, 849–859.

Buckner, J.H., Ziegler, S.F., 2008. Functional analysis of FOXP3. Ann. N. Y. Acad. Sci. 1143, 151–169.

Burchill, M.A., Yang, J., Vogtenhuber, C., Blazar, B.R., Farrar, M.A., 2007. IL-2 receptor beta-dependent STAT5 activation is required for the development of Foxp3$^+$ regulatory T cells. J. Immunol. 178, 280–290.

Carrier, Y., Yuan, J., Kuchroo, V.K., Weiner, H.L., 2007. Th3 cells in peripheral tolerance. I. Induction of Foxp3-positive regulatory T cells by Th3 cells derived from TGF-beta T cell-transgenic mice. J. Immunol. 178, 179–185.

Chen, G.Y., Chen, C., Wang, L., Chang, X., Zheng, P., Liu, Y., 2008. Cutting edge: broad expression of the FoxP3 locus in epithelial cells: a caution against early interpretation of fatal inflammatory diseases following *in vivo* depletion of FoxP3-expressing cells. J. Immunol. 180, 5163–5166.

Chen, W., Konkel, J.E., 2010. TGF-beta and 'adaptive' Foxp3(+) regulatory T cells. J. Mol. Cell Biol. 2, 30–36.

Chen, X., Hamano, R., Subleski, J.J., Hurwitz, A.A., Howard, O.M., Oppenheim, J.J., 2010. Expression of costimulatory TNFR2 induces resistance of CD4$^+$FoxP3$^-$ conventional T cells to suppression by CD4$^+$FoxP3$^+$ regulatory T cells. J. Immunol. 185, 174–182.

Ebelt, K., Babaryka, G., Frankenberger, B., Stief, C.G., Eisenmenger, W., Kirchner, T., Schendel, D.J., Noessner, E., 2009. Prostate cancer lesions are surrounded by FOXP3$^-$, PD-1$^+$ and B7-H1$^+$ lymphocyte clusters. Eur. J. Cancer 45, 1664–1672.

Fantini, M.C., Becker, C., Monteleone, G., Pallone, F., Galle, P.R., Neurath, M.F., 2004. Cutting edge: TGF-beta induces a regulatory phenotype in CD4$^+$CD25$^-$ T cells through Foxp3 induction and down-regulation of Smad7. J. Immunol. 172, 5149–5153.

Floess, S., Freyer, J., Siewert, C., Baron, U., Olek, S., Polansky, J., Schlawe, K., Chang, H.D., Bopp, T., Schmitt, E., Klein-Hessling, S., Serfling, E., Hamann, A., Huehn, J., 2007. Epigenetic control of the foxp3 locus in regulatory T cells. PLoS Biol. 5, e38.

Fontenot, J.D., Rudensky, A.Y., 2005. A well adapted regulatory contrivance: regulatory T cell development and the forkhead family transcription factor Foxp3. Nat. Immunol. 6, 331–337.

Fu, S., Zhang, N., Yopp, A.C., Chen, D., Mao, M., Zhang, H., Ding, Y., Bromberg, J.S., 2004. TGF-beta induces Foxp3$^+$ T-regulatory cells from CD4$^+$ CD25$^-$ precursors. Am. J. Transplant. 4, 1614–1627.

Fujii, H., Arakawa, A., Kitoh, A., Miyara, M., Kato, M., Kore-Eda, S., Sakaguchi, S., Miyachi, Y., Tanioka, M., Ono, M., 2010. Perturbations of both non-regulatory and regulatory FOXP3(+) T cells in patients with malignant melanoma. Br. J. Dermatol.

Fujimoto, M., Nakano, M., Terabe, F., Kawahata, H., Ohkawara, T., Han, Y., Ripley, B., Serada, S., Nishikawa, T., Kimura, A., Nomura, S., Kishimoto, T., Naka, T., 2011. The influence of excessive IL-6 production *in vivo* on the development and function of Foxp3$^+$ regulatory T cells. J. Immunol. 186, 32–40.

Gates, J.D., Clifton, G.T., Benavides, L.C., Sears, A.K., Carmichael, M.G., Hueman, M.T., Holmes, J.P., Jama, Y.H., Mursal, M., Zacharia, A., Ciano, K., Khoo, S., Stojadinovic, A., Ponniah, S., Peoples, G.E., 2010. Circulating regulatory T cells (CD4$^+$CD25$^+$FOXP3$^+$) decrease in breast cancer patients after vaccination with a modified MHC class II HER2/neu (AE37) peptide. Vaccine 28, 7476–7482.

Gupta, S., Joshi, K., Wig, J.D., Arora, S.K., 2007. Intratumoral FOXP3 expression in infiltrating breast carcinoma: its association with clinicopathologic parameters and angiogenesis. Acta Oncol. 46, 792–797.

Hancock, W.W., Ozkaynak, E., 2009. Three distinct domains contribute to nuclear transport of murine Foxp3. PLoS One 4, e7890.

Hansmann, L., Schmidl, C., Boeld, T.J., Andreesen, R., Hoffmann, P., Rehli, M., Edinger, M., 2010. Isolation of intact genomic DNA from FOXP3-sorted human regulatory T cells for epigenetic analyses. Eur. J. Immunol. 40, 1510–1512.

Harbuz, R., Lespinasse, J., Boulet, S., Francannet, C., Creveaux, I., Benkhelifa, M., Jouk, P.S., Lunardi, J., Ray, P.F., 2010. Identification of new FOXP3 mutations and prenatal diagnosis of IPEX syndrome. Prenat. Diagn. 30, 1072–1078.

Hodi, F.S., O'Day, S.J., McDermott, D.F., Weber, R.W., Sosman, J.A., Haanen, J.B., Gonzalez, R., Robert, C., Schadendorf, D., Hassel, J.C., Akerley, W., van den Eertwegh, A.J., Lutzky, J., Lorigan, P., Vaubel, J.M., Linette, G.P., Hogg, D., Ottensmeier, C.H., Lebbe, C., Peschel, C., Quirt, I., Clark, J.I., Wolchok, J.D., Weber, J.S., Tian, J., Yellin, M.J., Nichol, G.M., Hoos, A., Urba, W.J., 2010. Improved survival with ipilimumab in patients with metastatic melanoma. N. Engl. J. Med. 363, 711–723.

Horwitz, D.A., Zheng, S.G., Wang, J., Gray, J.D., 2008. Critical role of IL-2 and TGF-beta in generation, function and stabilization of Foxp3$^+$CD4$^+$ Treg. Eur. J. Immunol. 38, 912–915.

Huber, S., Stahl, F.R., Schrader, J., Luth, S., Presser, K., Carambia, A., Flavell, R.A., Werner, S., Blessing, M., Herkel, J., Schramm, C., 2009. Activin a promotes the TGF-beta-induced conversion of CD4$^+$CD25$^-$ T cells into Foxp3$^+$ induced regulatory T cells. J. Immunol. 182, 4633–4640.

Huter, E.N., Punkosdy, G.A., Glass, D.D., Cheng, L.I., Ward, J.M., Shevach, E.M., 2008. TGF-beta-induced Foxp3$^+$ regulatory T cells rescue scurfy mice. Eur. J. Immunol. 38, 1814–1821.

Inoue, N., Watanabe, M., Morita, M., Tomizawa, R., Akamizu, T., Tatsumi, K., Hidaka, Y., Iwatani, Y., 2010. Association of functional polymorphisms related to the transcriptional level of FOXP3 with prognosis of autoimmune thyroid diseases. Clin. Exp. Immunol. 162, 402–406.

Janson, P.C., Winerdal, M.E., Marits, P., Thorn, M., Ohlsson, R., Winqvist, O., 2008. FOXP3 promoter demethylation reveals the committed Treg population in humans. PLoS One 3, e1612.

Jung, D.J., Jin, D.H., Hong, S.W., Kim, J.E., Shin, J.S., Kim, D., Cho, B.J., Hwang, Y.I., Kang, J.S., Lee, W.J., 2010. Foxp3 expression in p53-dependent DNA damage responses. J. Biol. Chem. 285, 7995–8002.

Karanikas, V., Speletas, M., Zamanakou, M., Kalala, F., Loules, G., Kerenidi, T., Barda, A.K., Gourgoulianis, K.I., Germenis, A.E., 2008. Foxp3 expression in human cancer cells. J. Transl. Med. 6, 19.

Katagiri, Y., Hozumi, Y., Kondo, S., 2006. Knockdown of Skp2 by siRNA inhibits melanoma cell growth in vitro and *in vivo*. J. Dermatol. Sci. 42, 215–224.

Katoh, H., Zheng, P., Liu, Y., 2010. Signalling through FOXP3 as an X-linked tumor suppressor. Int. J. Biochem. Cell Biol. 42, 1784–1787.

Kaur, G., Goodall, J.C., Jarvis, L.B., Hill Gaston, J.S., 2010. Characterisation of Foxp3 splice variants in human CD4$^+$ and CD8$^+$ T cells–identification of Foxp3Delta7 in human regulatory T cells. Mol. Immunol. 48, 321–332.

Kawamoto, K., Pahuja, A., Hering, B.J., Bansal-Pakala, P., 2010. Transforming growth factor beta 1 (TGF-beta1) and rapamycin synergize to effectively suppress human T cell responses via upregulation of FoxP3$^+$ Tregs. Transpl. Immunol. 23, 28–33.

Kim, C.H., 2009. FOXP3 and its role in the immune system. Adv. Exp. Med. Biol. 665, 17–29.

Kim, H.P., Leonard, W.J., 2007. CREB/ATF-dependent T cell receptor-induced FoxP3 gene expression: a role for DNA methylation. J. Exp. Med. 204, 1543–1551.

Kiniwa, Y., Miyahara, Y., Wang, H.Y., Peng, W., Peng, G., Wheeler, T.M., Thompson, T.C., Old, L.J., Wang, R.F., 2007. CD8$^+$ Foxp3$^+$ regulatory T cells mediate immunosuppression in prostate cancer. Clin. Cancer Res. 13, 6947–6958.

Komatsu, N., Hori, S., 2007. Full restoration of peripheral Foxp3$^+$ regulatory T cell pool by radioresistant host cells in scurfy bone marrow chimeras. Proc. Natl. Acad. Sci. U S A 104, 8959–8964.

Ladoire, S., Arnould, L., Mignot, G., Coudert, B., Rebe, C., Chalmin, F., Vincent, J., Bruchard, M., Chauffert, B., Martin, F., Fumoleau, P., Ghiringhelli, F., 2011. Presence of Foxp3 expression in tumor cells predicts better survival in HER2-overexpressing breast cancer patients treated with neoadjuvant chemotherapy. Breast Cancer Res. Treat. 125, 65–72.

Lal, G., Bromberg, J.S., 2009. Epigenetic mechanisms of regulation of Foxp3 expression. Blood 114, 3727–3735.

Lal, G., Yin, N., Xu, J., Lin, M., Schroppel, S., Ding, Y., Marie, I., Levy, D.E., Bromberg, J.S., 2011. Distinct inflammatory signals have physiologically divergent effects on epigenetic regulation of foxp3 expression and treg function. Am. J. Transplant. 11, 203–214.

Lal, G., Zhang, N., van der Touw, W., Ding, Y., Ju, W., Bottinger, E.P., Reid, S.P., Levy, D.E., Bromberg, J.S., 2009. Epigenetic regulation of Foxp3 expression in regulatory T cells by DNA methylation. J. Immunol. 182, 259–273.

Lan, Y., Tang, X.S., Qin, J., Wu, J., Qin, J.M., 2010. Association of transcription factor FOXP3 gene polymorphism with genetic susceptibility to systematic lupus erythematosus in Guangxi Zhuang population. Zhonghua YiXue YiChuanXue ZaZhi 27, 433–436.

Lee, S.M., Gao, B., Fang, D., 2008. FoxP3 maintains Treg unresponsiveness by selectively inhibiting the promoter DNA-binding activity of AP-1. Blood 111, 3599–3606.

Li, B., Greene, M.I., 2008. Special regulatory T-cell review: FOXP3 biochemistry in regulatory T cells–how diverse signals regulate suppression. Immunology 123, 17–19.

Li, R., Perez, N., Karumuthil-Melethil, S., Prabhakar, B.S., Holterman, M.J., Vasu, C., 2007. Enhanced engagement of CTLA-4 induces antigen-specific $CD4^{+}CD25^{+}Foxp3^{+}$ and $CD4^{+}CD25^{-}$ TGF-beta 1^{+} adaptive regulatory T cells. J. Immunol. 179, 5191–5203.

Li, W., Wang, L., Katoh, H., Liu, R., Zheng, P., Liu, Y., 2011. Identification of a tumor suppressor Relay between the FOXP3 and the Hippo pathways in breast and prostate cancers. Cancer Res..

Lin, Y.C., Lee, J.H., Wu, A.S., Tsai, C.Y., Yu, H.H., Wang, L.C., Yang, Y.H., Chiang, B.L., 2011. Association of single-nucleotide polymorphisms in FOXP3 gene with systemic lupus erythematosus susceptibility: a case-control study. Lupus 20, 137–143.

Liu, Y., Wang, Y., Li, W., Zheng, P., 2009. Activating transcription factor 2 and c-Jun-mediated induction of FoxP3 for experimental therapy of mammary tumor in the mouse. Cancer Res. 69, 5954–5960.

Liu, Y., Zheng, P., 2007. FOXP3 and breast cancer: implications for therapy and diagnosis. Pharmacogenomics 8, 1485–1487.

Lopes, J.E., Soper, D.M., Ziegler, S.F., 2007. Foxp3 is required throughout the life of a regulatory T cell. Sci. STKE ., pe36.

Luo, X., Zhang, Q., Liu, V., Xia, Z., Pothoven, K.L., Lee, C., 2008. Cutting edge: TGF-beta-induced expression of Foxp3 in T cells is mediated through inactivation of ERK. J. Immunol. 180, 2757–2761.

Mahic, M., Henjum, K., Yaqub, S., Bjornbeth, B.A., Torgersen, K.M., Tasken, K., Aandahl, E.M., 2008. Generation of highly suppressive adaptive CD8(+)CD25(+)FOXP3(+) regulatory T cells by continuous antigen stimulation. Eur. J. Immunol. 38, 640–646.

Mahmoud, S.M., Paish, E.C., Powe, D.G., Macmillan, R.D., Lee, A.H., Ellis, I.O., Green, A.R., 2010. An evaluation of the clinical significance of FOXP3(+) infiltrating cells in human breast cancer. Breast Cancer Res. Treat..

Maitra, U., Davis, S., Reilly, C.M., Li, L., 2009. Differential regulation of Foxp3 and IL-17 expression in CD4 T helper cells by IRAK-1. J. Immunol. 182, 5763–5769.

Mantel, P.Y., Ouaked, N., Ruckert, B., Karagiannidis, C., Welz, R., Blaser, K., Schmidt-Weber, C.B., 2006. Molecular mechanisms underlying FOXP3 induction in human T cells. J. Immunol. 176, 3593–3602.

Marie, J.C., Letterio, J.J., Gavin, M., Rudensky, A.Y., 2005. TGF-beta1 maintains suppressor function and Foxp3 expression in $CD4^{+}CD25^{+}$ regulatory T cells. J. Exp. Med. 201, 1061–1067.

Merlo, A., Casalini, P., Carcangiu, M.L., Malventano, C., Triulzi, T., Menard, S., Tagliabue, E., Balsari, A., 2009. FOXP3 expression and overall survival in breast cancer. J. Clin. Oncol. 27, 1746–1752.

Miyara, M., Sakaguchi, S., 2011. Human FoxP3(+)CD4(+) regulatory T cells: their knowns and unknowns. Immunol. Cell Biol.

Miyara, M., Yoshioka, Y., Kitoh, A., Shima, T., Wing, K., Niwa, A., Parizot, C., Taflin, C., Heike, T., Valeyre, D., Mathian, A., Nakahata, T., Yamaguchi, T., Nomura, T., Ono, M., Amoura, Z., Gorochov, G., Sakaguchi, S., 2009. Functional delineation and differentiation dynamics of human $CD4^{+}$ T cells expressing the FoxP3 transcription factor. Immunity 30, 899–911.

Moon, C., Kim, S.H., Park, K.S., Choi, B.K., Lee, H.S., Park, J.B., Choi, G.S., Kwan, J.H., Joh, J.W., Kim, S.J., 2009. Use of epigenetic modification to induce FOXP3 expression in naive T cells. Transplant. Proc. 41, 1848–1854.

Ochs, H.D., Gambineri, E., Torgerson, T.R., 2007. IPEX, FOXP3 and regulatory T-cells: a model for autoimmunity. Immunol. Res. 38, 112–121.

Ono, M., Yaguchi, H., Ohkura, N., Kitabayashi, I., Nagamura, Y., Nomura, T., Miyachi, Y., Tsukada, T., Sakaguchi, S., 2007. Foxp3 controls regulatory T-cell function by interacting with AML1/Runx1. Nature 446, 685–689.

Ouaked, N., Mantel, P.Y., Bassin, C., Burgler, S., Siegmund, K., Akdis, C.A., Schmidt-Weber, C.B., 2009. Regulation of the foxp3 gene by the Th1 cytokines: the role of IL-27-induced STAT1. J. Immunol. 182, 1041–1049.

Owen, C.J., Jennings, C.E., Imrie, H., Lachaux, A., Bridges, N.A., Cheetham, T.D., Pearce, S.H., 2003. Mutational analysis of the FOXP3 gene and evidence for genetic heterogeneity in the immunodysregulation, polyendocrinopathy, enteropathy syndrome. J. Clin. Endocrinol. Metab. 88, 6034–6039.

Polansky, J.K., Kretschmer, K., Freyer, J., Floess, S., Garbe, A., Baron, U., Olek, S., Hamann, A., von Boehmer, H., Huehn, J., 2008. DNA methylation controls Foxp3 gene expression. Eur. J. Immunol. 38, 1654–1663.

Polansky, J.K., Schreiber, L., Thelemann, C., Ludwig, L., Kruger, M., Baumgrass, R., Cording, S., Floess, S., Hamann, A., Huehn, J., 2010. Methylation matters: binding of Ets-1 to the demethylated Foxp3 gene contributes to the stabilization of Foxp3 expression in regulatory T cells. J. Mol. Med. 88, 1029–1040.

Roncarolo, M.G., Gregori, S., 2008. Is FOXP3 a bona fide marker for human regulatory T cells? Eur. J. Immunol. 38, 925–927.

Rose, A.E., Wang, G., Hanniford, D., Monni, S., Tu, T., Shapiro, R.L., Berman, R.S., Pavlick, A.C., Pagano, M., Darvishian, F., Mazumdar, M., Hernando, E., Osman, I., 2011. Clinical relevance of SKP2 alterations in metastatic melanoma. Pigment Cell Melanoma Res. 24, 197–206.

Ruan, Q., Kameswaran, V., Tone, Y., Li, L., Liou, H.C., Greene, M.I., Tone, M., Chen, Y.H., 2009. Development of Foxp3(+) regulatory t cells is driven by the c-Rel enhanceosome. Immunity 31, 932–940.

Rubio-Cabezas, O., Minton, J.A., Caswell, R., Shield, J.P., Deiss, D., Sumnik, Z., Cayssials, A., Herr, M., Loew, A., Lewis, V., Ellard, S., Hattersley, A.T., 2009. Clinical heterogeneity in patients with FOXP3 mutations presenting with permanent neonatal diabetes. Diabetes Care 32, 111–116.

Sadlon, T.J., Wilkinson, B.G., Pederson, S., Brown, C.Y., Bresatz, S., Gargett, T., Melville, E.L., Peng, K., D'Andrea, R.J., Glonek, G.G., Goodall, G.J., Zola, H., Shannon, M.F., Barry, S.C., 2010. Genome-wide identification of human FOXP3 target genes in natural regulatory T cells. J. Immunol. 185, 1071–1081.

Samanta, A., Li, B., Song, X., Bembas, K., Zhang, G., Katsumata, M., Saouaf, S.J., Wang, Q., Hancock, W.W.,

Shen, Y., Greene, M.I., 2008. TGF-beta and IL-6 signals modulate chromatin binding and promoter occupancy by acetylated FOXP3. Proc. Natl. Acad. Sci. U S A 105, 14023–14027.

Schneider, A., Rieck, M., Sanda, S., Pihoker, C., Greenbaum, C., Buckner, J.H., 2008. The effector T cells of diabetic subjects are resistant to regulation via $CD4^+$ $FOXP3^+$ regulatory T cells. J. Immunol. 181, 7350–7355.

Schramm, C., Huber, S., Protschka, M., Czochra, P., Burg, J., Schmitt, E., Lohse, A.W., Galle, P.R., Blessing, M., 2004. TGF-beta regulates the $CD4^+CD25^+$ T-cell pool and the expression of Foxp3 *in vivo*. Int. Immunol. 16, 1241–1249.

Simonetta, F., Chiali, A., Cordier, C., Urrutia, A., Girault, I., Bloquet, S., Tanchot, C., Bourgeois, C., 2010. Increased CD127 expression on activated $FOXP3^+CD4^+$ regulatory T cells. Eur. J. Immunol. 40, 2528–2538.

Smith, E.L., Finney, H.M., Nesbitt, A.M., Ramsdell, F., Robinson, M.K., 2006. Splice variants of human FOXP3 are functional inhibitors of human $CD4^+$ T-cell activation. Immunology 119, 203–211.

Soligo, M., Camperio, C., Caristi, S., Scotta, C., Del Porto, P., Costanzo, A., Mantel, P.Y., Schmidt-Weber, C.B., Piccolella, E., 2011. CD28 costimulation regulates FOXP3 in a RelA/NF-kappaB-dependent mechanism. Eur. J. Immunol. 41, 503–513.

Spatz, A., Borg, C., Feunteun, J., 2004. X-chromosome genetics and human cancer. Nat. Rev. Cancer 4, 617–629.

Takaki, H., Ichiyama, K., Koga, K., Chinen, T., Takaesu, G., Sugiyama, Y., Kato, S., Yoshimura, A., Kobayashi, T., 2008. STAT6 Inhibits TGF-beta1-mediated Foxp3 induction through direct binding to the Foxp3 promoter, which is reverted by retinoic acid receptor. J. Biol. Chem. 283, 14955–14962.

Thornton, A.M., Korty, P.E., Tran, D.Q., Wohlfert, E.A., Murray, P.E., Belkaid, Y., Shevach, E.M., 2010. Expression of Helios, an Ikaros transcription factor family member, differentiates thymic-derived from peripherally induced $Foxp3^+$ T regulatory cells. J. Immunol. 184, 3433–3441.

Torgerson, T.R., Linane, A., Moes, N., Anover, S., Mateo, V., Rieux-Laucat, F., Hermine, O., Vijay, S., Gambineri, E., Cerf-Bensussan, N., Fischer, A., Ochs, H.D., Goulet, O., Ruemmele, F.M., 2007. Severe food allergy as a variant of IPEX syndrome caused by a deletion in a noncoding region of the FOXP3 gene. Gastroenterology 132, 1705–1717.

Valdman, A., Jaraj, S.J., Comperat, E., Charlotte, F., Roupret, M., Pisa, P., Egevad, L., 2010. Distribution of $Foxp3^-$, $CD4^-$ and $CD8^-$positive lymphocytic cells in benign and malignant prostate tissue. APMIS 118, 360–365.

Vang, K.B., Yang, J., Mahmud, S.A., Burchill, M.A., Vegoe, A.L., Farrar, M.A., 2008. IL-2, -7, and -15, but not thymic stromal lymphopoeitin, redundantly govern $CD4^+Foxp3^+$ regulatory T cell development. J. Immunol. 181, 3285–3290.

Veldman, C., Pahl, A., Hertl, M., 2009. Desmoglein 3-specific T regulatory 1 cells consist of two subpopulations with differential expression of the transcription factor Foxp3. Immunology 127, 40–49.

Venuprasad, K., Huang, H., Harada, Y., Elly, C., Subramaniam, M., Spelsberg, T., Su, J., Liu, Y.C., 2008. The E3 ubiquitin ligase Itch regulates expression of transcription factor Foxp3 and airway inflammation by enhancing the function of transcription factor TIEG1. Nat. Immunol. 9, 245–253.

Wang, C., Lisanti, M.P., Liao, D.J., 2011. Reviewing once more the c-myc and Ras collaboration: converging at the cyclin D1-CDK4 complex and challenging basic concepts of cancer biology. Cell Cycle 10, 57–67.

Wang, D., Zhang, H., Liang, J., Gu, Z., Zhou, Q., Fan, X., Hou, Y., Sun, L., 2010a. $CD4^+$ $CD25^+$ but not $CD4^+$ $Foxp3^+$ T cells as a regulatory subset in primary biliary cirrhosis. Cell Mol. Immunol. 7, 485–490.

Wang, L., Liu, R., Ribick, M., Zheng, P., Liu, Y., 2010b. FOXP3 as an X-linked tumor suppressor. Discov. Med. 10, 322–328.

Wang, L., Liu, R., Li, W., Chen, C., Katoh, H., Chen, G.Y., McNally, B., Lin, L., Zhou, P., Zuo, T., Cooney, K.A., Liu, Y., Zheng, P., 2009. Somatic single hits inactivate the X-linked tumor suppressor FOXP3 in the prostate. Cancer Cell 16, 336–346.

Wang, R., Han, G., Wang, J., Song, L., Chen, G., Xu, R., Yu, M., Qian, J., Shen, B., Li, Y., 2007. The role of STAT3 in antigen-IgG inducing regulatory CD4(+)Foxp3(+)T cells. Cell Immunol. 246, 103–109.

Wildin, R.S., Freitas, A., 2005. IPEX and FOXP3: clinical and research perspectives. J. Autoimmun. 25 (Suppl), 56–62.

Wu, Y., Borde, M., Heissmeyer, V., Feuerer, M., Lapan, A.D., Stroud, J.C., Bates, D.L., Guo, L., Han, A., Ziegler, S.F., Mathis, D., Benoist, C., Chen, L., Rao, A., 2006. FOXP3 controls regulatory T cell function through cooperation with NFAT. Cell 126, 375–387.

Xu, L., Kitani, A., Stuelten, C., McGrady, G., Fuss, I., Strober, W., 2010. Positive and negative transcriptional regulation of the Foxp3 gene is mediated by access and binding of the Smad3 protein to enhancer I. Immunity 33, 313–325.

Yokokawa, J., Cereda, V., Remondo, C., Gulley, J.L., Arlen, P.M., Schlom, J., Tsang, K.Y., 2008. Enhanced functionality of $CD4^+$CD25(high)$FoxP3^+$ regulatory T cells in the peripheral blood of patients with prostate cancer. Clin. Cancer Res. 14, 1032–1040.

Zhang, F., Meng, G., Strober, W., 2008. Interactions among the transcription factors Runx1, RORgammat and Foxp3 regulate the differentiation of interleukin 17-producing T cells. Nat. Immunol. 9, 1297–1306.

Zheng, Q., Xu, Y., Liu, Y., Zhang, B., Li, X., Guo, F., Zhao, Y., 2009. Induction of Foxp3 demethylation increases regulatory $CD4^+CD25^+$ T cells and prevents the occurrence of diabetes in mice. J. Mol. Med. 87, 1191–1205.

Zheng, S.G., Wang, J.H., Stohl, W., Kim, K.S., Gray, J.D., Horwitz, D.A., 2006. TGF-beta requires CTLA-4 early after T cell activation to induce FoxP3 and generate adaptive $CD4^+CD25^+$ regulatory cells. J. Immunol. 176, 3321–3329.

Zheng, Y., Josefowicz, S., Chaudhry, A., Peng, X.P., Forbush, K., Rudensky, A.Y., 2010. Role of conserved non-coding DNA elements in the Foxp3 gene in regulatory T-cell fate. Nature 463, 808–812.

Zhou, L., Lopes, J.E., Chong, M.M., Ivanov II, , Min, R., Victora, G.D., Shen, Y., Du, J., Rubtsov, Y.P., Rudensky, A.Y., Ziegler, S.F., Littman, D.R., 2008a. TGF-beta-induced Foxp3 inhibits T(H)17 cell differentiation by antagonizing RORgammat function. Nature 453, 236–240.

Zhou, X., Kong, N., Zou, H., Brand, D., Li, X., Liu, Z., Zheng, S.G., 2010. Therapeutic potential of TGF-beta-induced CD4(+) Foxp3(+) regulatory T cells in autoimmune diseases. Autoimmunity.

Zhou, Z., Song, X., Li, B., Greene, M.I., 2008b. FOXP3 and its partners: structural and biochemical insights into the regulation of FOXP3 activity. Immunol. Res. 42, 19–28.

Ziegler, S.F., 2006. FOXP3: of mice and men. Annu. Rev. Immunol. 24, 209–226.

Zorn, E., Nelson, E.A., Mohseni, M., Porcheray, F., Kim, H., Litsa, D., Bellucci, R., Raderschall, E., Canning, C., Soiffer, R.J., Frank, D.A., Ritz, J., 2006. IL-2 regulates FOXP3 expression in human $CD4^+CD25^+$ regulatory T cells through a STAT-dependent mechanism and induces the expansion of these cells *in vivo*. Blood 108, 1571–1579.

Zuo, T., Liu, R., Zhang, H., Chang, X., Liu, Y., Wang, L., Zheng, P., 2007a. FOXP3 is a novel transcriptional repressor for the breast cancer oncogene SKP2. J. Clin. Invest. 117, 3765–3773.

Zuo, T., Wang, L., Morrison, C., Chang, X., Zhang, H., Li, W., Liu, Y., Wang, Y., Liu, X., Chan, M.W., Liu, J.Q., Love, R., Liu, C.G., Godfrey, V., Shen, R., Huang, T.H., Yang, T., Park, B.K., Wang, C.Y., Zheng, P., 2007b. FOXP3 is an X-linked breast cancer suppressor gene and an important repressor of the HER-2/ErbB2 oncogene. Cell 129, 1275–1286.